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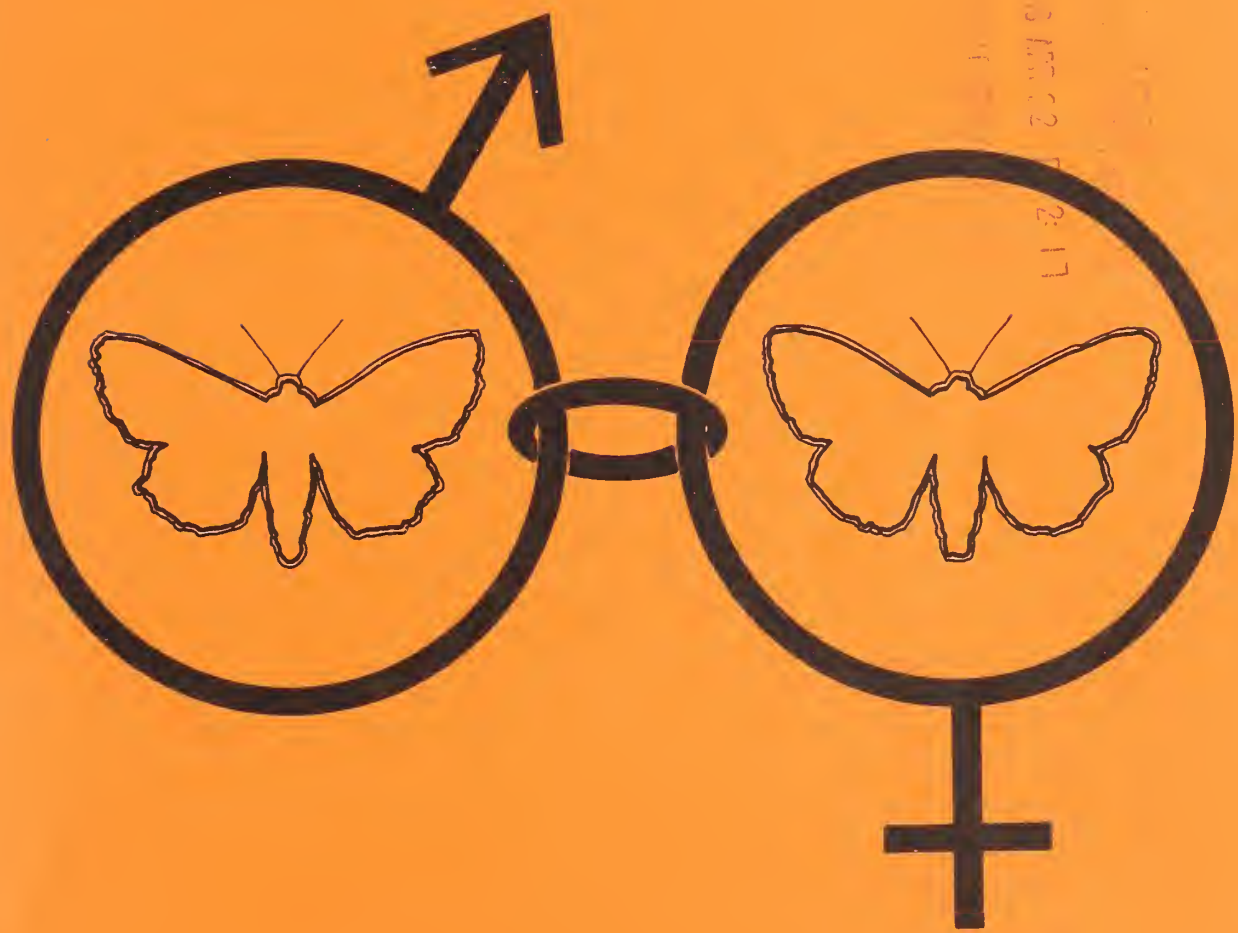
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INSECT ATTRACTANTS, BEHAVIOR, AND BASIC BIOLOGY
RESEARCH LABORATORY

Gainesville, Florida

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SECOND SEMI-ANNUAL REPORT - 1978



SCIENCE AND EDUCATION ADMINISTRATION
U. S. Department of Agriculture
Southern Region
Florida - Antilles Area

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ANNUAL REPORT

1978

Insect Attractants, Behavior, and Basic Biology

Research Laboratory

USDA-SEA, Southern Region, Florida-Antilles Area

P.O. Box 14565

Gainesville, Florida 32604

In cooperation with the Departments of

Entomology and Nematology

and

Agricultural Engineering

University of Florida

Because of a shortage of clerical assistance we were unable to prepare this report in the usual format. Instead we are utilizing the annual report that was prepared to meet USDA requirements. The number codes refer to specific budgeted programs, and are identified for each section.

This progress report includes tentative results of research not sufficiently completed to justify general release. Such findings when adequately confirmed will be released through established channels. Therefore, this report is not intended for publication and should not be referred to in literature citations.

Research Progress: NRP 20250 - Work reported herein is related to some reported for NRP 20259, 20302 and 20620

A₁ New and improved principles and practices of insect control based on selective disruption of their growth, development, and reproduction.

1. Progress Report:

(7602-20250-004)

- (a) To study the behavioral quality of colonized relative to wild-type insects.

Colonies of Heliothis backcross (H. virescens x H. subflexa, females progeny x male H. virescens) and H. subflexa are being maintained. Tests are in progress to determine if larval cannibalism is an important factor in the laboratory rearing process. Preliminary indications are that larvae of backcrosses are not cannibalistic when provided an ample supply of artificial diet; H. subflexa shows cannibalistic characteristics that favor the male over the female by 2:1 and the hybrid males tend to destroy the females at a ratio of 3:1.

The flight propensity (measured as a response to an overhead light source) of laboratory-reared Anastrepha suspensa males and females of different ages was determined and both sexes are most phototactic when they are 1 and 5 days old. Seven generations of selecting flies that responded to the light produced flies that are more phototactic than the controls.

(7602-20250-004 Cont'd.)

Irradiation had very little detrimental effect on flight ability of Anastrepha suspensa determined on a flight mill or with an electronic stroboscope, but it severely affected the pre-copulatory signal sound. Wild flies reared from rose apple or guava flew farther and spent more time in flight than laboratory flies reared on bagasse.

A new vibration-sensitive actograph system was invented and used to measure the relative activity of laboratory-reared and wild-type cabbage looper moths. In addition, the life histories of F₁-F₁₀ generations of the two populations were compared by recording the number of spermatophores transferred and viable eggs produced per mated female, the proportion of larvae surviving to pupation, and average pupal weights. The wild-type moths were found to be more active, but "strains" were reproductively equivalent.

The Mediterranean fruit fly is being used to test three colonization strategies. Larvae collected from peaches on Maui, Hawaii, were used to establish colonies that are being maintained with natural stimuli, eased into the established rearing regime by progressively removing stimuli, or forced directly into production. The survival and reproductive rates of the three populations are being measured from the F₁ to ca. F₁₀ generations in an attempt to identify primary selection pressures.

- (b) Determine if a tachinid, Eucelatoria sp. is capable of strong flight.

No progress

- (c) Determine the effect of cage size on the flight capability of A. suspensa males and females.

A colony of flies established at this laboratory has been kept in 32-cm cubical cages. A second colony will be established from the original one and kept in a significantly larger (walk-in) cage. The flight capability of the 2 colonies will be studied for several successive generations of flies.

(7602-20250-004 Cont'd.)

- (d) Determine the feasibility of using the electromyogram method of monitoring muscular activity in quality control.

No progress

- (e) To continue efforts on in vitro rearing of Apanteles marginiventris.

No progress

- (f) To further develop and test the visual sensitivity assessment method for monitoring quality of the laboratory reared insects.

Visual and spectral sensitivity of medflies from Hawaii reared under severe, moderate, and low stress conditions from wild stock for continuing generations under low larval density conditions were not statistically different. Sterilizing doses of irradiation of pupae produced adults with measurable reduction in visual sensitivity.

The oriental fruit fly was relatively more sensitive to the UV and red wavelengths than medflies. Colonized oriental fruit flies in Hawaii were visually less sensitive than wild flies. Diet and/or genetic divergence in the oriental fly population may account for the measured differences in wild and lab flies.

The mutant yellow-eyed melon fly was 14 to 170 times more sensitive to light stimuli than the normal eye color lab fly. There is a high probability that the mutant fly would not successfully reproduce in the field.

Medfly adults reared on diets with high density of larvae had reduced visual sensitivity compared to those from low larval density diets or wild medflies. Shipping and/or handling of pupae and sterilizing doses of irradiation further reduced the visual sensitivity of lab reared medflies tested in Wädenswil, Switzerland.

(7602-20250-004 Cont'd.)

New Work

To examine the potential of pre-release acclimation for improving performance of Lepidoptera reared for release.

Acclimation of soybean looper moths in the laboratory or outside for 2, 24, or 36-48 h did not affect the number of moths that dispersed from the released cages or that were trapped in blacklight or electrocutor grid traps. Thus, caged moths provided with food and water can be kept in the laboratory or held outside as long as 4 days after emergence before they are released in field experiments without affecting their ability to disperse and respond to baited traps in the field.

(7602-20250-005)

- (a) To detect and partition certain genetic phenomena, such as drift, inbreeding and selection, which may occur in mass-reared populations of the Mediterranean fruit fly.

A study, in cooperation with the Hawaiian Fruit Flies Investigations Laboratory, Honolulu, is in progress. The effects of three different methods of initiating new laboratory strains are being studied. Data on life history parameters and changes in allozyme allele frequencies are being collected.

(7602-20250-007)

- (a) To examine the effects of low temperature on the endocrine function of Indian meal moths.

Indian meal moth larvae were reared at temperatures of 30°C, 23° and 18°C. Mitochondria prepared from larvae reared at the lower temperatures had enzymatic alterations comparable to mitochondria treated with juvenile hormones (JH). The enzymatic alterations in the mitochondria were not intensified in insects reared at 18°C compared to those reared at 23°C. However, the addition of JH to 18°C mitochondria did intensify the enzymatic effects, suggesting that JH was not mediating the effect of the lower rearing temperatures on mitochondria.

(7602-20250-007 Cont'd.)

- (b) To elucidate the neuroendocrine control of secretion of growth and development hormones.

Corpora allata were dissected from last instar larvae of Galleria mellonella and cultured in vitro in a modified Grace's tissue culture medium. The glands remained healthy for incubation periods up to 3 weeks. However, isotopic precursors of JH were not converted into recoverable JH in vitro.

- (c) To determine the mode of action of ecdysone and other growth regulators on chitin synthesis.

Biochemical methods were used to assess the quantitative effects of beta-ecdysone on chitin biosynthesis in wing discs incubated with 0.5 μ C of C^{14} -glucosamine for the final 24 h of culture. Imaginal discs of P. interpunctella responded to increasing concentrations of β -ecdysone with increased chitin synthesis. The threshold is between 0.01 and 0.1 μ g/ml of hormone (2×10^{-8} M to 2×10^{-7} M). These data represent the first demonstration of quantitative biosynthesis of chitin by developing tissue in vitro in relation to varying amounts of hormone. Inhibition studies showed that protein synthesis during the β -ecdysone-dependent period was necessary for chitin synthesis. This system thus lends itself to a detailed investigation of the hormonal control of chitin biosynthesis.

- (d) To further define the biochemical basis for differential resistance observed among citrus varieties and species to attack by the Caribbean fruit fly. To assist plant breeders by characterizing resistant lines in terms of specific chemical and morphological features conferring resistance.

Bioassays were conducted in the laboratory to determine the relative susceptibility of various citrus cultivars (Temple oranges, March white grapefruit, Thompson pink grapefruit, plus Eureka and Lisbon lemons) to attack by the Caribbean fruit fly. Both types of lemons proved immune to attack, even when overripe, while the grapefruit were susceptible to a degree increasing with fruit senescence. Oranges were intermediate in susceptibility, and were most susceptible when senescent. Difference in susceptibility correlated with differences

(7602-20250-007 Cont'd.)

in peel oil abundance and composition, with lemons possessing the most peel oil per sq. cm and the most toxic oil. Senescence-related changes have not yet been explained.

A₂ New and improved principles and practices of insect control based on their behavior and ecology.

1. Progress Report:

(7602-20250-001)

- (a) To isolate and identify the pheromones of Heliothis zea, Manduca sexta, Anticarsia gemmatalis, and several species of armyworms.

Preliminary bioassays and field tests indicate the aldehydes proposed as H. zea and H. virescens pheromones by the Beltsville laboratory are active. Work is continuing to clearly define the pheromone chemistry and related behavior of H. zea, H. virescens, H. subflexa, and hybrids of the latter two species.

Improved biological and electrophysiological assays have been developed for the other species, active fractions have been isolated, and several components have been tentatively identified.

The pheromone of the beet armyworm has been isolated and crude pheromone is being collected for purification identification. Preliminary chemical characterization and laboratory bioassays indicate that the pheromone consists of at least 2 compounds.

A bioassay system has been developed for analyzing the reproductive behavior and identifying the primary female sex pheromone of the velvetbean caterpillar. A controlled environment chamber was renovated, an observation tunnel constructed and instrumented, and pre-conditioning protocols devised to provide for minimal variance in insect performance. The system has been used to determine the optimum concentration of female extract required to elicit male pre-mating behavior.

- (b) To complete the identification and synthesis of the white peach scale and navel orangeworm pheromones.

The white peach scale pheromone was identified as (R,Z)-3,9-dimethyl-6-isopropenyl-3,9-decadien-1-ol propionate. The pheromone was synthesized and the synthetic compound was very attractive to wild males in the field when compared to virgin females of the species.

(7602-20250-001 Cont'd.)

The four possible geometrical isomers of the navel orangeworm pheromone, 11,13-hexadecadienal, were synthesized and purified. Identification of the Z,Z-isomer as the pheromone was made based on biological (laboratory) activity. Purification of milligram amounts of this material permitted a preliminary field evaluation. The Z,Z-isomer was synthesized de novo by a route that provided that isomer in >99.5% geometrical purity.

- (c) To further develop analytical techniques to aid in the separation and identification of microgram or smaller quantities of biologically active natural products.

High resolution glass capillary GC columns have been further developed and improved and a variety of stationary phases have been investigated. Columns with efficiencies near the calculated theoretical value have been prepared and isomeric pheromone mixtures that previously could not be resolved have been separated. For example, all 4 isomers of the navel orangeworm pheromone, a conjugated diene system, can be completely resolved. It is now possible to determine the exact isomeric mixture produced by an insect with as little as 2 ng of pheromone obtained from one insect.

- (d) To develop efficient syntheses for isolated and identified pheromones.

A synthetic procedure has been developed for the preparation of (E,Z)-3,13-octadecadien-1-ol acetate, the sex attractant of the lesser peachtree borer, free of contamination by the Z,Z isomer, a powerful inhibitor for lesser peachtree borers. This procedure does not involve the use of high performance liquid chromatography to purify the pheromone and thus remove this bottleneck to making the pheromone more readily available for large scale field studies.

In the course of the development of this synthetic procedure, the stereochemistry of the dissolving metal reduction of acetylenic alcohols was thoroughly investigated and the conditions which control this stereochemistry were delineated.

(7602-20250-001 Cont'd.)

A study of the preparations and reductions of β -halo-trifluoroacetates and β -bistrifluoroacetates was completed. These materials can be obtained stereospecifically from olefins or their epoxides and are potentially useful derivatizing agents for GLC analysis. Their reductions can be made to proceed stereospecifically either to revert to the initial olefin geometry or to invert that geometry. This is a very useful tool for the synthesis of insect pheromones containing an olefinic link.

A method of inverting epoxide geometry has been tentatively found. The ability to deoxygenate epoxides stereospecifically to olefins increases the synthetic utility of this reaction sequence.

Eight chemicals have been synthesized as part of a project to develop pheromone mimics with the objective being the replacement of expensive, difficult to acquire synthetic pheromones with drastically less expensive and more readily available chemicals.

- (e) To develop and evaluate formulations to dispense pheromones and mating disruptants of loopers, noctuids and other pests of row crops, the Japanese beetle, and insects affecting peaches.

Hercon® and Conrel® dispensers were investigated as releasers for the peachtree borer pheromone, (Z,Z)-3, 13-octadecadien-1-ol acetate, in field tests. The release rates of this pheromone, from both dispensers, were measured periodically after aging in the field for 1 to six months. Additionally, capture of male PTB in traps baited with these same dispensers of various ages was monitored. At the end of the study the quantity of pheromone remaining in the dispensers was analyzed. The release rates of pheromone from both dispenser types were extremely low, and less than 1% of the pheromone evaporated in 5 months. However, trap captures decreased significantly after 2 to 3 months, and the amount of pheromone present in the dispensers at the end of the study could not be correlated with the release rate. Measured release rates and trap captures correlated well. Further work to develop better pheromone dispensers is needed.

(7602-20250-001 Cont'd.)

Carboaset and Conrel® (hollow fiber) formulations of the soybean looper pheromone were field-tested as mating disruptants. Carboaset released the pheromone too fast to be suitable for field-crops use in Florida. The hollow fiber system was more suitable and tests of commercial equipment for dispensing the fibers established feasibility for field crop use. Tests in peach orchards were deferred until equipment and formulations have been refined.

The emission rate of Z9TDF formulated in Conrel fibers was measured at 24°C and 35°C. Although the emission rate at the high temperature was initially 2.5 x higher than at 24°C, the emission rates were equal after 5 days. Emission of Z9TDF was at its lowest by the 12th day and remained there for 24 more days. All of the disruptant was emitted. The emission rate of Z9TDF from 1/8-in. and 1/4-in wide Hercon® strips was also measured. The emission rate from the 1/8-in. strips was slightly higher initially, but became the same as for the 1/4-in. strip after 3 days. Thereafter, the emission rates for both strips declined steadily for 18 days until all of the Z9TDF was emitted.

Cooperating scientists from BARC set up equipment in a corn field to sample the atmosphere for Z9TDF, and background samples were collected. However, the low insect population precluded application of the mating disruptant until the cooperators had to move to another project. Analysis of the background samples showed the test area was entirely satisfactory.

Leather pieces or plastic vials as pheromone dispensers were found to attract the greatest number of lesser peachtree borer males for a longer time period than the conventionally used rubber septa.

- (f) To investigate the chemical nature of the oviposition deterrent produced by the female apple maggot fly, Rhagoletis pomonella.

(7602-20250-001 Cont'd.)

The oviposition-detererring pheromone is highly polar, methanol and water soluble, stable, and may contain a volatile and an essentially non-volatile component. Its chromatographic behavior on gel-permeation columns indicates its molecular weight is less than 1500. Purification of the pheromone is continuing in cooperation with Dr. Prokopy, University of Massachusetts, Amherst, who is performing bioassays. A postdoctoral associate has been hired to conduct chemical investigations in Gainesville and additional technicians to conduct bioassays have been hired in Amherst.

(7602-20250-004)

- (a) To study the energetics of pheromones and other chemical cues, their relationship to other environmental factors, and to the responses elicited.

A Fourier transform interferometer has been modified to test 3-cm audio modulated radio frequency, UV and blue wavelengths so that they can be utilized to pump the scents. Coherent infrared emission has been detected from the scents as predicted. In all cases the coded coherent emission shifts with temperature and concentration. Coded coherent Raman, Stokes, and anti-Stokes wavelengths have been plotted for the cabbage looper pheromone (sex scent), benzaldehyde (plant scent), and human breath (mosquito attractant). In all cases the coherent radiation was predicated based on nonlinear and waveguide theory.

- (b) To examine the process of olfaction by morphological, nuerophysiological, behavioral and biochemical techniques.

Measurements of the important morphological features on the antennae of I. ni have been made. Various analyses demonstrate that quantitative mathematical models can be made of these features. From these models the surface area of the various types of sensilla can be calculated. The surface area's importance to olfactory sensitivity has been emphasized previously.

(7602-20250-004 Cont'd.)

Research into a new behavioral parameter that affects the behavioral threshold to pheromone application continues. A method of measuring sensitivity to pheromones using a newly developed model has been achieved. This will result in greater precision and economy of time. Because a second chemical component of the cabbage looper sex pheromone recently has been identified, the effects of this component on the typical attraction threshold was investigated. The second component appears to increase upwind orientation but not chemokinesis. It appears that the insect can discriminate between as little as 2-3% of the second component in (Z)-7-dodecen-1-ol acetate, and that decrements in attraction occur on either side of the optimum.

Over 500 non-parametric statistical correlations of behavioral response vs. neurophysiological response (single unit) to 17 different chemicals have been accomplished. It appears that some neurophysiological parameters can be correlated with behavior.

Membrane marker enzyme reactions were determined from a fraction isolated from chemosensory sensilla on the antenna of the cabbage looper. Both the method of isolating the antennal membrane fraction and the results of the enzyme assays indicated that the fraction included dendritic membranes from primary olfactory receptor cells. Two techniques, in addition to affinity-labeling techniques for measuring pheromone and isomers binding to protein receptors were developed to demonstrate pheromone-receptor binding.

The esterases from the antenna were isolated and partially purified. Enzyme kinetics of the hydrolysis of the pheromone and related chemicals revealed specificity for the natural pheromone, this indicates that hydrolytic enzymes may be involved in the process of pheromone olfactory transduction.

(7602-20250-004 Cont'd.)

- (c) To improve insect rearing through improved techniques.

Large re-usable containers have been constructed and tested for rearing the fall armyworm. The containers were designed to increase production efficiency by reducing the amount of labor required to rear larvae and harvest pupae. However, microbial contamination must be controlled before these new materials and techniques can be adopted.

- (d) Determine the most efficient spectral bandwidths for attracting the peachtree borer, the yellowjacket, the stable fly, fruit flies, and other species.

Yellowjacket traps painted buttercup yellow and baited with feeding attractants captured more workers and queens than traps painted other colors; however, between October-December, traps painted fluorescent saturn yellow captured more workers than traps painted fluorescent arc yellow, enamel buttercup yellow, or unpainted.

Based on spectral sensitivity characteristics of the medfly, a set of 5 color traps was selected for testing at several Mediterranean Sea area locations through the cooperation of Swiss scientists and members of the International Organization for Biological Control. The spectral sensitivity of wild medflies from each trapping location was measured to determine what differences might exist between local populations that could serve as a population identification factor. Yellow traps identified as #2037 and Boller (color formulation) were the most effective colors for attracting medflies. Tabulation of spectral sensitivity data for the various populations is not complete at this time.

In cooperation with staff of the Insects Affecting Man and Animals Laboratory and manufacturers, we are continuing our efforts to identify the specific wavelengths of light reflection from panels (AIsynite) responsible for fly attraction. Present spectral reflectance and behavioral response data indicate that reflections in the 380-410 nm region are important in the attraction process. Development of panels with improved reflections in these wavelengths and field testing are progressing.



(7602-20250-004 Cont'd.)

- (e) To identify and describe the elements of behavioral sequences in noctuid moths.

Velvetbean caterpillar females tend to orient to each other and aggregate while releasing pheromone. During the subsequent courship sequence, stimulated males apparently exchange chemical and tactile cues with calling females. A male approaches from an anteriodorsal position, extends its femoral setae, and assumes a tent-line posture over the dorsum of the female. They then rotate to the typical lepidopteran tandem position if intromission is effected. Considerable variation exists in the timing and duration of each element in these behavior patterns and, for mating to be accomplished, the actions must proceed in succession.

(7602-20250-005)

- (a) To study the effects of geographical and seasonal variation in pheromone response on the population structure of clearwing moths.

Allozyme studies were carried out on clearwing moths of the genus Podotesia. The early and late flight species, P. syringae and P. aureocincta respectively, are clearly distinct at the genetic level although their temporal distributions overlap slightly in Florida. A similar study of the lesser peachtree borer revealed no genetic differences between males captured in traps baited with the normal pheromone, (E,Z)-3,13-octadecadien-1-ol acetate, and traps baited with the Z,Z isomer.

The seasonal abundance of the lesser peachtree borer and peachtree borer was determined in north central Florida. Lesser peachtree borers were present in the field from February-December; peachtree borers were frequently present from August-November.



(7602-20250-005 Cont'd.)

The seasonal occurrence of 20 species of Sesiidae was determined in Alachua and Marion Counties in north central Florida during 1975-78. Sticky traps baited with EZ- or ZZ-ODDA, EZ- or ZZ-ODDOH, or various combinations of them attracted 15 species of 25 currently recognized Sesiidae plus 5 species not reported before: Synanthedon decipiens, Synanthedon fatifera, Carmenta bassiformis, Carmenta suffusata, and Alcathoe carolinensi.

- (b) To investigate the feasibility of determining the region of origin of introduced Mediterranean fruit flies.

A worldwide survey of genetic variation in this species is in progress. Preliminary results indicate that there is little allozyme variation in introduced populations. Samples from Israel, Hawaii and Central America appear to share the same alleles. Hawaii is indicated as the source of the Central American infestation at the present time.

- (c) To utilize pheromones to monitor and suppress insect populations.

University of Florida cooperators were found to facilitate future studies to suppress the tomato pinworm under commercial conditions via pheromone permeation.

Female-baited traps were used for the third consecutive year to monitor the white peach scale. It now appears that pheromone-baited traps can be used to identify major flights of male scales.

Adult populations in the corn earworm and fall armyworm were monitored throughout the spring and summer in traps and around fields of sweet corn using pheromone traps (sticky, cone, electric grid) and 15-W blacklight traps. The pheromone traps were baited with virgin females (CEW or FAW) and synthetic pheromone (FAW).



(7602-20250-005 Cont'd.)

Populations of both species were extremely low early in the season (March-June) but increased in number during mid-to-late summer (July-September). Pheromone traps effectively indicated peak populations of adult CEW and FAW. Blacklight traps were effective for monitoring the seasonal occurrence of the CEW, but they were ineffective for monitoring the FAW.

Pheromone-baited traps placed ca. 30 m apart around two 2.7 ha tobacco fields appeared to act as a barrier to H. virescens males entering the fields until tobacco plants began to form flower buds (button stage). Significantly more moths were captured in traps outside the field than in an equal number of traps located within the fields.

Cone traps baited with (Z)-11-hexadecenal: (Z)-9-tetradecenal (16:1) were used to monitor male tobacco budworm moth activity in post-harvest tobacco fields and 3 weed patches consisting primarily of Florida beggarweed, Desmodium tortuosum. There were 2 distinct periods of moth activity; the 1st peak occurred on August 30 (ca. 2.5 TBW/trap/night) and the 2nd peak (ca. 1 TBW/trap/night) ca. 5 weeks later. There was no significant difference in the dates of peak moth captures in post-harvest tobacco fields and beggarweed.

Conrel® hollow fibers filled with (Z)-9-tetradecen-1-ol formate (Z9TDF) was applied by helicopter to plots of sweet corn (2.1 acres ea) at the rate of 15, 30, and 60 g/acre. The disruptive effect of Z9TDF on pheromone communication in the corn earworm (CEW) was measured by placing female-baited traps near the center of each plot. The deposition pattern of the fibers on the plants was also determined. The 60 and 30 g rates gave 100% disruption for 7 and 5 days, respectively; the 15 g rate was ineffective. The number of fibers deposited on plants was directly proportional to the application rate. The distribution pattern showed 64, 21, and 15% of the fibers were deposited on the top, middle, and bottom of the plants, respectively. Sixty-four percent of the fibers deposited on the corn remained intact 8 days or longer following 1 overhead irrigation.



(7602-20250-005 Cont'd.)

New Pherocon-1C sticky traps captured the most lesser peachtree borer males, and 26-week-old traps captured the fewest numbers except for traps (8 weeks old) baited before with the peachtree borer sex pheromone (ZZ-ODDA) which captured the fewest number of males. New and aged traps baited with fresh pheromone captured more lesser peachtree borer males than new or aged traps baited with aged pheromone. Trap catches were optimized when the traps and pheromone were replaced every 3 weeks.

- (d) Determine and monitor parasite populations attacking Heliothis virescens.

The predominant parasite species of H. virescens in tobacco during the growing season was Cardiochiles nigriceps, followed by Temelucha sp. and Apanteles marginiventris.

Parasites were identified from Heliothis spp. larvae collected from post-harvest tobacco and Florida beggarweed in Alachua Co., Florida. Cardiochiles nigriceps (Vierick) (Hymenoptera: Braconidae) built up to populations infesting ca. 14% of the hosts on beggarweed and ca. 25% on volunteer tobacco which grew from plowed-under stalks in late September and October. Archytas marmoratus (Townsend) (Diptera: Tachinidae) was first collected from H. virescens on August 3, 1978, and by October nearly 50% of the Heliothis larvae from beggarweed were parasitized. No other parasite species were collected in significant numbers.

- (e) Obtain data to develop a predictive egg deposition model for Heliothis virescens and H. zea populations in Florida.

MOTHZV-2, a computer simulation model of Heliothis zea and H. virescens population dynamics, was obtained from USDA personnel in Texas and adapted to the University of Florida computer system. During the spring and summer of 1978, data were gathered on the capture of corn earworms in blacklight traps, weather, crop phenology, and egg and larval populations in 2

(7602-20250-005 Cont'd.)

varieties of sweet corn. The corn was subjected to the usual intensive insect control program practiced in Florida. Analysis of the data are underway, and the model is being modified for use under Florida conditions.

- (f) To conceive, design, and construct equipment and devices for the evaluation of insect behavior and for monitoring insect populations.

Cone-shaped traps constructed of 3.2 mm mesh hardware cloth and treated with 8% active solution of permethrin to kill captured moths did not significantly increase the total catch of H. virescens males. However, when lab-reared moths were released into the treated traps, with the exception of those that escaped (ca. 5%), almost 100% of the moths were killed by the permethrin.

Current research on battery-powered electrocutor grid traps was completed early in FY-78. Maintenance problems have required continual improvement in the photo-switch used with these devices. The design of a low-cost microprocessor (μ P) controlled data acquisition system was instigated. Such a data acquisition system has been designed that will cost about \$750 (10% or less than costs of commercially available units) that will be of general use in most types of biological research. A general program (software) allowing use of the system by researchers, untrained in the use of a μ P, is being developed.

- (g) To develop new insect pheromones.

Supporting field and laboratory bioassays established the validity of the sex pheromone identified from the white peach scale. The rhythm of female sex pheromone release was established and a manuscript is in preparation.

Evaluation of the male-produced pheromone of the cabbage looper was not conducted.

(7602-20250-005 Cont'd.)

- (h) To study genetic aspects of host plant selection in Heliothis virescens, H. subflexa, and their inter-specific hybrids in the laboratory and field.

No progress to report. Study underway at present time.

- (j) To determine the behavioral basis for mating disruption via permeation of the air with sex pheromone.

Disruption of mating communication with sex pheromones is influenced by the stratum in which an insect flies and mates. Mating communication is more easily disrupted in the peachtree borer which flies and mates near or at the ground than in the lesser peachtree borer which flies and mates in the upper portions of the trees.

- (k) Determine those factors responsible for the regulation of the sex ratio of Apanteles marginiventris, a primary parasite of the fall armyworm.

Light quality was the principal factor affecting the sex ratio during the mating and ovipositional periods. Light intensity was also important but played a lesser role. The sex ratio was adversely affected during the mating period if the proportion of males was less than 33%. The results of this research were transferred to the parasite rearing operation resulting in an increase in the proportion of females from 20 to 60%.

- (l) Determine the location of the chemical stimuli responsible for the initiation of host finding behavior in Apanteles marginiventris and develop reliable bioassays.

No progress. This research will be started in April 1979.

- (m) Determine the effect of parasitization by Apanteles marginiventris on food consumption, and rate of growth of fall armyworm larvae.



(7602-20250-005 Cont'd.)

Parasitization by A. marginiventris reduced the food consumption of fall armyworm larvae by 155% when compared to unparasitized larvae. Parasitization reduced the time spent in the larval stage by 5.5 days. All parasitized FAW larvae were killed in the 3rd instar.

New Work

- (a) Correct and extend the model for control of Heliothis virescens through hybrid male sterility.

The present model demonstrated that the ratio of re-released backcross hybrids to native H. virescens remains constant in a closed population. It was also shown that the release ratio necessary to achieve extinction of a closed population is a function of the number of females that a male can inseminate and the population growth rate. Other biological aspects of this system were also investigated.

- (b) Determine the relationship between pheromone trap captures of Heliothis virescens and larval population levels in cultivated crops.

Significant correlations were found between pheromone-baited trap catches of male H. virescens moths and (1) larval infestations and (2) damage levels in tobacco fields when weekly trap catches were compared with larval and damage counts made 1 and 2 weeks later. There was also a significant correlation between larval infestation and plant damage involving counts made the same week.

- (c) To investigate population dynamics of Heliothis spp. on weed species and post-harvest tobacco.

Sweep samples (15" diam net) and manual collections were made for Heliothis spp. larvae from post-harvest tobacco and Florida beggarweed, Desmodium tortuosum, in tobacco fields in Alachua Co., Florida. Peak larval populations followed peak pheromone-trap captures. The ratio of



(7206-20250-005 New Work Cont'd.)

H. zea to H. virescens decreased from 75% (tobacco) and 50% (beggarweed) in July to 0% in October. Throughout the season H. virescens outnumbered H. zea 3:1 on beggarweed. Populations on beggarweed reached ca. 3.5 larvae/sweep (over 100 young larvae/10' plant). Preliminary results indicate tobacco hornworms, Manduca sexta, compete heavily with Heliothis larvae on tobacco regrowth after harvest.

- (d) Establish the exotic parasite Eiphosoma vitticole on the fall armyworm in south Florida.

The parasite has been identified and released from quarantine after importation from Bolivia. Successful laboratory rearing techniques were developed to obtain sufficient numbers for a release program. Releases were started in February 1979.



Principal Accomplishments and Significance

Quality Assays Developed For USA/Mexico Mediterranean Fruit Fly Program

Elimination of threatening populations of medfly from areas on and south of the border between Mexico and Guatemala is being undertaken by a joint USDA/Mexico program. Nearly one billion flies per week will be reared in southern Mexico, sterilized, and distributed over infested areas. Efficacy and efficiency of control is entirely dependent upon continuous production of behaviorally effective flies. Personnel of the Insect Attractants, Behavior, and Basic Biology Research Laboratory, together with scientists from SEA-Hawaii, APHIS, and IAEA and their Swiss consultant have devised and tested assays that promptly detect and identify deficiencies in mass-produced medflies. Simplified versions of these tests have been developed and personnel of the joint program trained in their use. This is the first case of provision of a complete quality control protocol to a sterile-release control program in advance of production inception.

White Peach Scale Pheromone Identified and Synthesized

The sex pheromone of the white peach scale was identified with less than 5 μg of the natural substance isolated from ca. 1 million females. The isolation and identification was accomplished with a combination of high resolution chromatographic and micro-spectroscopic techniques previously developed in this laboratory. The synthesized pheromone attracted large numbers of wild males of the species when it was tested in the field in competition with females. This is the second scale insect pheromone identified and is important in increasing our basic knowledge of this group of insects of worldwide importance. In an IPM program for peaches, the white peach scale is a key member of the pest complex that must be managed, and the pheromone will facilitate detection of very low populations.

Exotic Parasites Shows Promise as Control Agent for Fall Armyworm

Eiphosoma vitticole is an exotic parasite of the fall armyworm which was imported into Florida from Bolivia in February 1978. Rearing procedures were developed which provide sufficient numbers of the parasite for release in south Florida, one of the few areas in the United States where the fall armyworm survives the winter. Parasite releases commenced in February 1979 and will continue through the spring. If the parasite becomes established, this



(Cont'd.)

would be the first step in a long-range program designed to suppress the fall armyworm population in the overwintering habitat thereby reducing the number of migrant moths invading areas farther north each spring and summer.

Pheromone Traps May Be Useful For Scheduling Control Measures For Tobacco Budworm

Cone-shaped traps constructed of screen-wire and baited with the tobacco budworm sex pheromone were used to monitor adult male populations throughout the 1978 tobacco growing season in Alachua County, Florida. Trap captures of male moths from the overwintered and F₁ generations were correlated with (1) larval infestations and (2) damage levels in tobacco fields. With refinements, captures of tobacco budworm moths in pheromone traps may be used in conjunction with other scouting techniques to apply control measures to tobacco and other crops such as cotton when needed rather than on an automatic schedule.

Mating Disruption Shows Promise For Control Of The Corn Earworm And Fall Armyworm In Sweet Corn

Sweet corn in Florida is treated 20-24 times per season with insecticides for control of the corn earworms and fall armyworm. (Z)-9-tetradecen-1-ol formate and (Z)-9-tetradecen-1-ol acetate effectively disrupt the mating process of the CEW and FAW, respectively, when evaporated into the atmosphere of corn fields. These materials are non-toxic to humans and other animals. When these mating disruptants are used simultaneously in conjunction with conventional control measures, recent research suggests that it may be possible to reduce the number of insecticide applications normally used for control of the CEW and FAW in sweet corn by 50% or more. This approach to insect control holds a special interest for aerial applicators because the materials are non-toxic, and they are applied at very low rates/acre (15-70 g formulated material). This allows the pilots more flying time because less time is spent in refilling spray tanks.

Research Progress: NRP 20620 - Work reported herein is related to some reported for NRP 20250 and NRP 20302

A. New and improved technology to reduce losses to insects and mites in storing and marketing agricultural products.

1. Progress Report:

(7602-20620-009)

- (a) To determine the optimum conditions for the use of pheromones in control and monitoring of stored product Lepidoptera (almond moth, Angoumois grain moth, Indian meal moth).

Sex pheromone traps were used to monitor the populations of the almond moth and Indian meal moth in 2 commercial peanut warehouses over a two-year period. The results showed that these traps make excellent tools for estimating population levels, timing fumigations, and determining the effectiveness of fumigations and insecticidal treatment. Similar traps were placed in two military food warehouses, one of which was thought to be free of insects. The traps discovered significant hidden populations of almond moth and Indian meal moth in both warehouses. The traps were able to pinpoint the locations of the infested commodities.

- (b) To establish an in vitro culture system for investigating the development and activity of the sex pheromone gland of the Indian meal moth.



Sex pheromone glands of adult and pharate adult female P. interpunctella were successfully maintained in tissue culture for up to 10 days in either chemically defined or modified Grace's medium. Pheromone bioassays of extracts prepared from cultured glands or extracts prepared from the rearing medium showed that more pheromone was recovered from extracts of modified Grace's medium than from chemically defined medium. Histological studies revealed that sex pheromone gland cells retained a normal appearance after 10 days in culture.

- (c) To identify a sex pheromone produced by male Indian meal moths, and to evaluate the effects of this pheromone on mating behavior.

A bioassay was developed to support identification of the male-produced sex pheromone of the Indian meal moth. Also, parameters of female response rhythm and male pheromone production rhythm were established. An electroantennogram (EAG) study determined that female antennae exhibit an electrical response to male wing gland extracts; the use of the EAG as a bioassay may be feasible.

- (d) To evaluate the synthetic pheromone of the navel orangeworm and to investigate the potential role of chemical compounds in mating communication.

Syntheses and laboratory bioassay of all four geometric isomers of 11,13-hexadecadienal were completed. Only the Z,Z isomer elicited pheromone responses, and only the Z,Z isomer co-chromatographed with the natural product on several GLC columns. Field tests, conducted in California, yielded data in total agreement with laboratory findings, i.e., only the Z,Z isomer was attractive to feral males. Approximately 10 g of isomerically pure (>99%) Z,Z were synthesized and formulated in polyvinyl chloride for field tests in 1979.



- (e) To investigate the reproductive patterns in stored-product Coleoptera.

Evidence was obtained that host-produced volatiles enhance oviposition by Lasioderma serricorne females on nonhost substrates. Bioassays were developed to monitor aggregation-arrestment behavior of L. serricorne females in response to chemicals that were extracted from paprika. Initial studies of feeding and oviposition by female Cylas formicarius elegantulus were completed. Evidence was obtained that extractable stimuli are present in the skin and/or periderm of sweet potato, but not the pith, that elicit feeding by female weevils. Evidence was also obtained that skin and/or periderm, but not the pith of sweet potato contains stimuli that elicit oviposition by mated female weevils.

- (f) To investigate population dynamics of red flour beetles under conditions similar to those of residual infestations in marketing channels.

Work was postponed pending review of progress, preparation of manuscript and location of suitable field site.

- (g) To evaluate the potential for integration of several target specific, environmentally safe pest management techniques using the almond moth as a model.

The parasite, Bracon hebetor concentrated 85% of its attacks upon almond moth larvae that feed inside of peanut shells. Therefore, application of insect growth regulators should increase parasitization by extending the feeding period of host larvae. Roughly a third of the paralyzed host larvae had no Bracon eggs, making the accumulation of host larvae that are paralyzed, but not yet utilized for offspring development, a vital part of host-parasite population dynamics.

The mate-seeking strategy of the almond moth that we aim to disrupt with a sex pheromone-permeated atmosphere was characterized by a stationary female



releasing pheromone and extensive male flight lasting a mean of 10 min. In a stepwise progression around the test room, males added one m^2 to area searched for each min of flight. Females were generally located rapidly once the male was within <1 m of the female location.

(7602-20620-010)

- (a) To examine the effects of low temperature on the endocrine function of Indian meal moths.

Indian meal moth larvae were reared at temperatures of 30°C, 23° and 18°C. Mitochondria prepared from larvae reared at the lower temperatures had enzymatic alterations comparable to mitochondria treated with juvenile hormone (JH). The enzymatic alterations in the mitochondria were not intensified in insects reared at 18°C compared to those reared at 23°C. However, the addition of JH to 18°C mitochondria did intensify the enzymatic effects, suggesting that JH was not mediating the effect of the lower rearing temperatures on mitochondria.

- (b) To elucidate the neuroendocrine control of secretion of growth and development hormones.

Corpora allata were dissected from last instar larvae of Galleria mellonella and cultured in vitro in a modified Grace's tissue culture medium. The glands remained healthy for incubation periods up to 3 weeks. However, isotopic precursors of JH were not converted into recoverable JH in vitro.

- (c) To determine the mode of action of ecdysone and other growth regulators on chitin synthesis.

Biochemical methods were used to assess the quantitative effects of beta-ecdysone on chitin biosynthesis in wing discs incubated with 0.5 μ C of C^{14} -glucosamine for the final 24 h of culture. Imaginal discs of P. interpunctella responded to increasing concentrations of β -ecdysone with increased chitin synthesis. The threshold is between 0.01 and 0.1 μ g/ml of hormone ($2 \times 10^{-8}M$ to $2 \times 10^{-7}M$). These data represent the

first demonstration of quantitative biosynthesis of chitin by developing tissue in vitro in relation to varying amounts of hormone. Inhibition studies showed that protein synthesis during the β -ecdysone-dependent period was necessary for chitin synthesis. This system thus lends itself to a detailed investigation of the hormonal control of chitin biosynthesis.

- (d) To evaluate the effectiveness of insect growth regulators in the control of stored-product Lepidoptera.

Several insect growth regulators, which were effective against several insect species, were found to be ineffective on the Indian meal moth except for RO 13-5223 which was totally effective at concentrations as low as 0.5 ppm in the diet.

Four other insect growth regulators (IGRs) were tested for efficacy in suppressing populations of almond moth, Ephestia cautella (Walker), in inshell peanuts. In laboratory tests, peanuts were sprayed with several concentrations of either hydroprene, methoprene, diflubenzuron, or Stauffer MV-678 and infested with 200 eggs of almond moths. Concentrations below 100 ppm of all IGRs except diflubenzuron (hydroprene, 5 ppm; methoprene, 25 ppm; MV-678, 90 ppm) completely suppressed adult emergence. These IGRs also reduced fecundity at lower concentrations. MV-678 at about 30 ppm completely inhibited oviposition. Residues of hydroprene, methoprene, and MV-678 were as effective as residues of malathion against the almond moth after at least 8 months of storage.

Principal Accomplishments and Significance

Recent studies on the utilization of insect growth regulators (IGRs) to control the almond moth on inshell peanuts suggest their high potential as an alternative to pesticides. In laboratory tests the IGRs, hydroprene, methoprene, and Stauffer MV-678 favorably compared with malathion for effectiveness in preventing adult moth emergence from samples of peanuts. Moreover, even after 8 months of storage hydroprene, methoprene, and MV-678 were shown to be more effective than malathion in preventing adult moth emergence. Reports of resistant populations of the almond moth to malathion suggest that other control agents need to be developed. IGRs, besides being easily formulated for application to the commodity with the same equipment used for malathion, also have other advantages, including very low vertebrate toxicity, short environmental persistence, and reduced or no harmful effects to the natural enemies of the almond moth. Application of this method of pest management to other commodities under post-harvest conditions is likely.

The six-year study to identify and synthesize the navel orangeworm pheromone has been successfully completed. The pheromone was identified as one of the isomers of 11-13,hexadecadienal based on spectral and chemical data gathered from less than 0.1 milligram of pheromone that was collected from 60,000 female moths. Syntheses and bioassay of the four possible isomers have shown that of the 4, only one, the Z-Z isomer, was biologically active. Its biological and chemical characteristics were identical to the female-produced natural pheromone. Field tests in almond orchards confirmed the attractiveness of synthetic pheromone to feral males. Traps baited with synthetic pheromone will be used to monitor navel orangeworm populations in almond orchards so that chemical controls can be more effectively timed. In addition, the pheromone holds promise for controlling this major pest of almonds by disrupting the communication (pheromone) system by which the male finds the female.

Research Progress: NRP 20302 - Work reported herein is related to some reported for NRP 20250 and 20620

A. Develop equipment and techniques to increase effectiveness of nonchemical control of insect pest.

1. Progress Report:

(7602-20302-002)

- (a) Determine if fruit flies (Tephritidae) can be identified as to species and geographic origin based upon analysis of the sounds they produce.

Studies were undertaken to compare the physical properties of the premating sound of Ceratitis capitata fruit flies from three widely separated geographical areas, Austria, Costa Rica, and Hawaii. Preliminary results indicate the medfly produces three distinct sounds prior to mating. These appear to signify calling, courtship and precopulation. The calling sound is composed of one continuous sound, the courtship sound is composed of pulses and pulse trains and the pre-copula is one short burst of sound. Also indicated is that the fundamental frequency of the calling sound and the pulse train duration of the courtship sound may be correlated with geographic origin.

The sounds recorded of Dacus spp. fruit flies in Australia and Hawaii were analyzed. The physical characteristics of each species were determined. There were differences in some aspects of the physical properties of all species tested. Promise for discernment of genetic variants was demonstrated.

- (b) Determine if the sounds produced by Ceratitis capitata fruit flies can be used as a measure of quality in mass-reared flies.

A cooperative project was initiated with IAEA, Vienna, Austria to determine if irradiation altered the normal pattern of the calling sound of Ceratitis capitata fruit flies. Preliminary results show an inverse relationship between the irradiation dose level and the pulse train duration (PTD) of the courtship portion of the calling sound. The higher the dosage level the shorter the PTD. Other physical factors of the calling sound are being analyzed. The results of this study will be incorporated in our quality control program.

- (c) Determine if the acoustical properties of the flight sounds of Lepidoptera can be used to identify species in flight.

Flight sounds of the corn earworm and cabbage looper are being recorded. However, no interpretations of these data are possible at this time.

- (d) Identify the characteristics of the sounds produced by the plum curculio that are important as behavior modifiers.

No progress

- (e) Determine if the acoustical properties of the plum curculio can be used as one measure of quality control during colonization.

This work has been delayed until additional personnel are available.

(7602-20302-003)

- (a) Determine the important physical factors involved in insect pheromone release, dispersion and the subsequent detection of this pheromone by insects in their natural habitat.

Mass-transport theory and behavioral studies were used to identify the important factors involved in the release of pheromone from a trap in a warehouse, the resultant dispersion of the pheromone, and its subsequent detection by the insects. From these studies a model was derived that describes the attraction of Plodia interpunctella to a trap in a warehouse. This work is being prepared for publication.

Instrumentation was purchased to begin collection of field data so that the model could be verified. Efforts were begun to interface the instrumentation to the university computer so that the field data can be statistically analyzed easily.

A model was derived that describes the deposition of pheromone onto an insect antenna. Experimental verification of the model is under way.

Principal Accomplishments and Significance

Studies were undertaken to characterize the sounds produced by the Mediterranean fruit fly. Characteristic sounds are produced, noticeably by males, as a precondition to mating. The objective was to determine if flies can be identified as to strain or geographic origin, and whether deleterious affects of rearing, irradiation, and handling can be detected in mass-rearing facilities. Three distinct sounds have been identified with presumptive behavioral roles. Correlation of physical patterns with geographic origin and with treatment are indicated.

Research Progress: NRP 20259 - Work reported herein is related to some reported for NRP 20250, 20302 and 20620.

A₂ New and improved principles and practices of insect control based on their behavior and ecology.

1. Progress Report:

(7602-20259-001)

- (a) To establish test plots on experimental and private farms for evaluation of mating disruptants.

Land for experimental corn plots was acquired through farmer cooperators.

- (b) To develop and evaluate formulations to dispense mating disruptants of the corn earworm and fall armyworm.

Conrel® hollow fibers filled with (Z)-9-tetradecen-1-ol format (Z9TDF) were applied by helicopter to plots of sweet corn (2.1 acres ea) at the rate of 15, 30, and 60 g/acre. The disruptive effect of Z9TDF on pheromone communication in the corn earworm (CEW) was measured by placing female-baited traps near the center of each plot. The deposition pattern of the fibers on the plants was also determined. The 60 and 30 g rates gave 100% disruption for 7 and 5 days, respectively; the 15 g rate was ineffective. The number of fibers deposited on plants was directly proportional to the application rate.

The distribution pattern showed 64, 21, and 15% of the fibers were deposited on the top, middle, and bottom of the plants, respectively. Sixty-four percent of the fibers deposited on the corn remained intact 8 days or longer following 1 overhead irrigation.

The emission rate of Z9TDF formulated in Conrel fibers was measured at 24°C and 35°C. Although the emission rate at the high temperature was initially 2.5 x higher than at 24°C, the emission rates were equal after 5 days. Emission of Z9TDF was at its lowest by the 12th day and remained there for 24 more days. All of the disruptant was emitted. The emission rate of Z9TDF from 1/8-in. and 1/4-in. wide Hercon® strips was also measured. The emission rate from the 1/8-in. strips was slightly higher initially, but became the same as for the 1/4-in. strip after 3 days. Thereafter, the emission rates for both strips declined steadily for 18 days until all of the Z9TDF was emitted.

Cooperating scientists from BARC set up equipment in a corn field to sample the atmosphere for Z9TDF, and background samples were collected. However, the low insect population precluded application of the mating disruptant until the cooperators had to move to another project. Analysis of the background samples showed the test area was entirely satisfactory.

- (c) To synthesize and evaluate disruptants chemically bound to polymers.

No progress

- (d) To monitor the seasonal occurrence of corn earworm and fall armyworm populations in sweet corn and other crops using pheromone and blacklight traps and other sampling techniques.

Adult populations in the corn earworm and fall armyworm were monitored throughout the spring and summer in and around fields of sweet corn using pheromone traps (sticky, cone, electric grid) and 15-W blacklight traps. The pheromone traps were baited with virgin females (CEW or FAW) and synthetic pheromone (FAW). Populations of both species were extremely low early in the season (March-June) but increased in number during mid-to-late summer (July-September). Pheromone traps effectively indicated peak populations of adult CEW and FAW. Blacklight traps were effective for monitoring the seasonal occurrence of the CEW, but they were ineffective for monitoring the FAW.

- (e) To incorporate population and climatic data into a predictive egg deposition model for Heliothis modified from a model developed in Texas for use in corn, cotton, and sorghum.

MOTHZV-2, a computer simulation model of Heliothis zea and H. virescens population dynamics, was obtained from USDA personnel in Texas and adapted to the University of Florida computer system. During the spring and summer of 1978, data were gathered on the capture of corn earworms in blacklight traps, weather, crop phenology, and egg and larval populations in 2 varieties of sweet corn. The corn was subjected to the usual intensive insect control program practiced in Florida. Analysis of the data are underway, and the model is being modified for use under Florida conditions.

Research Progress: NRP 20853 - Work reported herein is related to some reported for NRP 20250.

- A₁ New and improved technologies for the control of insects and other arthropods that attack people and their belongings to improve human health and safety, recreational comfort, and agricultural production.

1. Progress Report:

(7602-20853-001)

To isolate, identify and synthesize the pheromone(s) produced by queens of the imported fire ant and to investigate the role that these and related compounds play in the communication and behavior of the imported fire ant.

The extracts of field-collected queens have been distilled and fractionated by liquid and gas chromatography. The distillation and chromatographic fractions were monitored for activity by two laboratory assays and by occasional field assays of fractions that showed activity in the lab. Concentration-activity curves have been constructed using both bioassays. Results indicate the existence of at least two volatile active substances. There also appears to be at least one substance of very low volatility which may be necessary to completely reconstruct the "queen response" by workers to surrogate queens.

Appendix 1 (1)

*Insect Attractants, Behavior, and Basic Biology
Research Laboratory*

*USDA-SEA, Southern Region, Florida-Antilles Area
1700 S.W. 23rd Drive at Archer Road
P.O. Box 14565
Gainesville, Florida 32604*

904-373-6701

FTS-947-7011

Derrell L. Chambers, Director

Ecology Research Group

- E. R. Mitchell, Research Entomologist; Ecology, Pheromones (Res. Leader)
 - W. W. Copeland, Agr. Research Technician
 - R. W. Hines, Agr. Research Technician
 - D. M. Jackson, Postdoctoral*
- T. R. Ashley, Research Entomologist; Ecology, Biological Control
 - R. D. Miller, Lab Technician*
 - W. G. Sercey, Biol. Lab. Technician
- M. D. Huettel, Research Entomologist; Genetics, Behavior
 - W. C. Gaddis, Biol. Lab. Technician
 - K. S. Woodburn, Lab. Technician*
- J. R. McLaughlin, Research Entomologist; Ecology, Pheromones
 - T. Krueger, Biol. Lab. Technician
 - R. R. Rutter, Biol. Lab. Technician
- F. C. Tingle, Research Entomologist; Ecology, Attractants
 - M. McKoy, Agr. Research Technician

*University of Florida, Dept. of Entomology and Nematology, Cooperating

Physiology Research Group

- H. Oberlander, Research Physiologist; Behavior, Endocrinology (Res. Leader)
C. E. Leach, Biol. Lab. Technician
S. Miller, Postdoctoral*
D. Nickle, Postdoctoral*
Asoka Srinivasan, Visiting Scientist
- J. A. Coffelt, Research Entomologist; Biology, Pheromones
W. T. McClellan, Lab. Technician*
- S. M. Ferkovich, Research Entomologist; Endocrinology
A. Harris, Lab. Technician*
F. VanEssen, Biol. Lab. Technician
- P. D. Greany, Research Entomologist; Physiology, Host-Parasite Relationships
S. Styer, Research Assistant*
- D. W. Hagstrum, Research Entomologist; Biology, Ecology
L. Davis, Lab. Technician*
- M. S. Mayer, Research Entomologist; Physiology, Pheromones
G. Lemire, Biol. Lab. Technician
- D. L. Silhacek, Research Chemist; Endocrinology, Metabolism
G. Whitmer, Lab. Technician*
- K. W. Vick, Research Entomologist; Biology, Pheromones
M. Sullivan, Biol. Lab. Technician

Chemistry Research Group

- J. H. Tumlinson, Research Chemist; Analytic Chemistry (Res. Leader)
M. Brennan, Phy. Science Technician
D. Culligan, Lab. Technician*
B. Dueben, Lab. Technician*
A. T. Proveaux, Phy. Science Technician
K. W. Chan, Research Associate*
J. R. Rocca, Lab. Technician*
- J. H. Cross, Research Chemist; Formulation Chemistry
- R. E. Doolittle, Research Chemist; Synthetic Chemistry
D. Parham, Lab. Technician*
- R. R. Heath, Chemist
J. R. Jordan, Lab. Technician*
- P. E. Sonnet, Research Chemist; Organic Chemistry



Ethology Research Group

- D. L. Chambers, Research Entomologist; Behavior, Physiology (Res. Leader)
T. C. Carlysle, Biol. Lab Technician
A. J. Hill, Agr. Res. Technician
- H. R. Agee, Research Entomologist; Audio Physiology
J. Davis, Biol. Lab. Technician
- P. S. Callahan, Research Entomologist; Radiation Physiology
- N. C. Leppla, Research Entomologist; Biology, Behavior, Rearing
S. Barrett, Lab. Technician*
C. W. Green, Lab. Technician*
R. H. Guy, Biol. Lab. Technician
J. Rye, Biol. Lab. Technician.
- J. L. Sharp, Research Entomologist; Movement, Distribution
J. D. James, Biol. Lab. Technician

Bioengineering Research Group

- J. C. Webb, Agricultural Engineer; Physical Attractants, Acoustics
(Acting Research Leader)
J. C. Benner, Jr., Electrical Technician
R. W. Mankin, Lab. Technician*
S. Masuda, Phy. Science Technician
- E. W. Hamilton, Research Entomologist: Bioengineering
F. L. Lee, Electrical Technician

Clerical Staff

- B. F. Brooke, Secretary Steno
E. S. Miller, Clerk Steno
E. S. Turner, Clerk Steno

PUBLICATION LIST

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INSECT ATTRACTANTS, BEHAVIOR AND BASIC BIOLOGY RESEARCH LABORATORY

Complete list available on request.
Reprints may be requested by number.

Address inquiries to:

*D. L. Chambers, Director
Insect Attractants, Behavior and
Basic Biology Research Laboratory
Agricultural Research
Science and Education Administration, USDA
P. O. Box 14565, Gainesville, FL 32604 USA*

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